PRINCIPLES OF GENE THERAPY IN ONCOLOGY

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Properties of a good drug

1. Safe
2. Effective
3. Stable
4. Synthetically feasible to produce
5. Soluble
6. Novel
If you have the drug you have to go through:

- in vitro testing
- animal testing

Phase I
Phase II clinical trials
Phase III
market
Number of approved new molecules for the treatment of cancer by the Food and Drug Administration.
Cancer Gene Therapy Trials (year approved/initiated)
What is Gene Therapy?

Gene therapy is a technique for introducing the genetic material of a gene in a patient that lacks that gene because of a mutation.
Genetic diseases:

**Type 1**: Single locus (gene) is defective and responsible for the disease, 100% heritable.

examples: Sickle cell anemia, Hypercholesterolemia, Cystic fibrosis

**Type 2**: Polygenic traits, <100% heritable, may be dependent on environmental factors and lifestyle.

examples: Heart disease, Cancer, Diabetes
<table>
<thead>
<tr>
<th>Indications</th>
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<td>Ocular diseases</td>
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Cancer Gene Therapy Trials (year approved/initiated)
A survey of gene transfer clinical trials | Most gene transfer clinical trials are conducted in the United States

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<td><strong>1579</strong></td>
<td></td>
<td><strong>1019</strong></td>
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Most gene-transfer trials are designed to assess only the safety of a particular gene-therapy approach (PHASE I). Few gene therapies are being assessed in PHASE II or PHASE III efficacy trials.

<table>
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<th>Phase</th>
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<tr>
<td>Phase I</td>
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<td>Phase I/II</td>
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<td><strong>1579</strong></td>
<td><strong>%</strong></td>
<td><strong>1019</strong></td>
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</table>
The first CGT trials –1991 (phase I)
Steven A. Rosenberg

Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor
Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF)
Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Interleukin-2 (IL-2)

First Phase III trials --1996
Randomized Multicenter Trial Comparing the Efficacy of Surgery, Radiation, and Injection of Murine Cells Producing Herpes Simplex Thymidine Kinase Vector Followed by Intravenous Ganciclovir Against the Efficacy of Surgery and Radiation in the Treatment of Newly Diagnosed, Previously Untreated Glioblastoma

A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme (Hum Gene Ther. 2000 Nov 20;11(17):2389-401.)
Gene therapy for malignant melanoma
Gendicine intratumoral injection combined with radiotherapy for advanced nasopharyngeal carcinoma

Gendicine intratumoral injection combined with radiotherapy for advanced cervical carcinoma

A Phase III, multi-center, open-label, randomized study to compare the Effectiveness and Safety of intratumoral administration of INGN-201 in combination with chemotherapy versus chemotherapy alone in 288 patients with recurrent Squamous Cell Carcinoma of the Head and Neck (SCCHN) - INGN-201 Trial 302

An international, randomised, double blind, placebo controlled, parallel group study to investigate whether TroVax?, added to first-line standard of care therapy, prolongs the survival of patients with locally advanced or metastatic clear cell renal adenocarcinoma. (TV3/001/06)

A Multicenter, Double Blind, Placebo controlled, Randomized study of TroVax? vs. Placebo in the First Line treatment of Patients with Metastatic Colorectal Cancer receiving chemo-based therapy ? EFC10528

Phase III study: infusion of donor lymphocytes transduced with the suicide gene gene HSV-TK after transplantation of allogenic T-depleted stem cells from a haploidentical donor in patients with haematological malignancies

Phase III studies in advanced metastatic prostate cancer patients using immunotherapy with an allogenenic prostate tumor cell vaccine, stably transduced with the human GM-CSF gene by non-pathogenic, replication defective, recombinant adeno-associated vira

A Phase III Randomized, Open-Label Study of CG1940/CG8711 Versus Docetaxel and Prednisone in Patients with Metastatic Hormone-Refractory Prostate Cancer who are Chemotherapy-Na?ve

A Phase III Randomized, Open-Label Study of Docetaxel in Combination with CG1940/CG8711 versus Docetaxel and Prednisone in Taxane-Na?ve Patients with Metastatic Hormone-Refractory Prostate Cancer with Pain
OPEN PHASE III GENE THERAPY TRIALS IN ONCOLOGY-2010

A Phase 3 Clinical Trial to Evaluate the Safety and Efficacy of Treatment with 2 mg Intralvesional Allovectin-7? Compared to Dacarbazine (DTIC) or Temozolomide (TMZ) in Subjects with Recurrent Metastatic Melanoma


A Phase III Randomized, Open-Label Study of CG1940 and CG8711 Versus Docetaxel and Prednisone in Patients with Metastatic Hormone-Refractory Prostate Cancer who are Chemotherapy-Na?ve. EuraCT: 2005-002738-36

A randomised efficacy trial of herpes simplex virus HSV1716 in recurrent glioblastoma
QUASAR V: A multi-centre randomised placebo-controlled trial of TroVax? vaccination in the adjuvant treatment of stage II and stage III colorectal cancer
A multicenter, double blind, placebo controlled, randomized study of TroVax vs placebo in the first line treatment of patients with metastatic colorectal cancer receiving standard of care EudraCT No:

A Randomized Phase 3 Clinical Trial to Evaluate the Efficacy and Safety of Treatment with OncoVEXGM-CSF Compared to Subcutaneously Administered GM-CSF In Previously Treated Melanoma Patients with Unresectable Stage IIIb, IIIc and IV Disease EudraCT No: 2008-006140-20

A Phase III Multi-Center, Open-Label, Randomized Study to Compare the Overall Survival and Safety off Bi-Weekly Intratumoral Administration of RPR/INGN 201 Versus Weekly Methotrexate in 240 Patients with Refractory Squamous Cell Carcinoma of the Head and Neck (SCCHN)

A Phase III, Multi-Center, Open-Label, Randomized Study to Compare the Effectiveness and Safety of Intratumoral Administration of RPR/INGN 201 in Combination with Chemotherapy Versus Chemotherapy Alone in 288 Patients with Recurrent Squamous Cell Carcinoma of the Head and Neck (SCCHN). Sponsor: Aventis Pharmaceuticals - Gencell Division
A Phase 3 Clinical Trial to Evaluate the Safety and Efficacy of Treatment with 2 mg Intralesional Allovectin-7 Compared to Dacarbazine (DTIC) or Temozolomide (TMZ) in Subjects with Recurrent Metastatic Melanoma

A Phase III Study of a PSA Vaccine in Androgen Ablation Refractory Prostate Cancer with Absence of Metastatic Disease and GM-CSF

Phase III Registration Study of Lucanix (TM), a TGF-β2 Antisense Gene-Modified, Allogeneic Tumor Cell Cocktail vs. Pemetrexed in Patients with Stages III/IV Non-Small Cell Lung Cancer

A Randomized, Controlled Phase III Trial of Replication-Competent Adenovirus-Mediated Suicide Gene Therapy in Combination with IMRT Versus IMRT Alone for the Treatment of Newly-Diagnosed Intermediate-Risk Prostate Cancer

A Randomized Phase 3 Clinical Trial to Evaluate the Efficacy and Safety of a Treatment with OncoVEXGM-CSF Compared to Subcutaneously Administered GM-CSF in Previously Treated Melanoma Patients with Unresectable Stage IIIb, IIIc and IV Disease
The problems of gene therapy of cancer

- Target
- Toxicity
- Targetting (Inefficient gene delivery)
  - Transduction efficiency
  - Metastatic nature of cancer
  - Immune response against vectors
Cancer gene therapy targets

Anti-angiogenesis
- Endostatin, angiostatin

T-Cell
- Vaccines
- Tumor antigens
- Cytokines
- Chemokines

Dendritic cell
- Hematopoietic
- Stem Cells

Drug Resistance Genes

Oncogenes
- p53
- p21
- Bcr-abl

Suicide Genes
- HSTK
- CD
- Bax
- caspases
<table>
<thead>
<tr>
<th>Gene type</th>
<th>Number</th>
<th>%</th>
<th>CGT-Number</th>
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<tr>
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<tr>
<td>Antigen</td>
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<tr>
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<td>0</td>
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<td>308</td>
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<td>Growth factor</td>
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<td>7.7</td>
<td>9</td>
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<tr>
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<tr>
<td>Gene type</td>
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<td>%</td>
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<tr>
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<td>--------</td>
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<td>------------</td>
</tr>
<tr>
<td>Marker</td>
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<tr>
<td>Oncogene regulator</td>
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<td>10</td>
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<td>Oncolytic virus</td>
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<tr>
<td>Porins, ion channels, transporters</td>
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<tr>
<td>Receptor</td>
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<td>5.6</td>
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<tr>
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<td>siRNA</td>
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<td>Tumor suppressor</td>
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<td>Total</td>
<td>1579</td>
<td>3.2</td>
<td>1019</td>
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Cancer gene therapy and immunotherapy trials
Gene therapy to protect haematopoietic cells from the toxic effects of chemotherapy. The aim of haematopoietic gene therapy is to insert a drug resistance (DR) vector into the chromosomal DNA of haematopoietic stem cells.

P-glycoprotein as a transmembrane drug efflux pump. The multidrug resistance gene *MDR1*, which encodes the cell-surface molecule P-glycoprotein (PGP), can confer resistance to a wide variety of drugs.
TARGET: Tumor suppressor genes

Cancer gene therapy by delivery of tumour-suppressor genes
- growth arrest
- apoptosis,
- unexpected bystander effects.
TARGET: Oncogenes

Antisense oligonucleotides selectively inhibit the synthesis of proteins by hybridizing with a target RNA transcript.
Delivery of agents that block oncogene (Onc) expression. These include genes that encode antisense oligonucleotides, which block oncogene expression, and ribozymes, which cleave oncogene transcripts.
Target: Cell killing (suicide gene delivery)

**Enzyme-prodrug combinations for suicide gene therapy**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Prodrug</th>
<th>Product</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>HSV-tk</td>
<td>Ganciclovir</td>
<td>Ganciclovir triphosphate</td>
<td>Blocks DNA synthesis</td>
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<tr>
<td>Cytosine deaminase</td>
<td>5-Fluorocytosine</td>
<td>5-Fluorouracil (5-FU)</td>
<td>Pyrimidine antagonist: blocks DNA and RNA synthesis</td>
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<tr>
<td>Nitroreductase</td>
<td>Nitrobenzyloxy carbonyl anthracyclines</td>
<td>Anthracyclines</td>
<td>DNA crosslinking</td>
</tr>
<tr>
<td>Carboxylesterase</td>
<td>CPT-11</td>
<td>SN38</td>
<td>Topoisomerase inhibitor</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>Cyclophosphamide</td>
<td>Phosphoramide mustard</td>
<td>DNA alkylating agent: blocks DNA synthesis</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase</td>
<td>6-Mercaptopurine-DR</td>
<td>6-Mercaptopurine</td>
<td>Purine antagonist: blocks DNA synthesis</td>
</tr>
</tbody>
</table>
Target: Cell killing (oncolysis-conditionally replicating viruses)

Conditionally replicating viruses. a | Mechanism of action. The viruses infect both normal and tumour cells, but can only replicate in tumour cells. The progeny then go on to kill surrounding tumour cells. b | Replication of a conditionally replicating virus, ONYX-015, in a cancer cell from a patient with head and neck cancer

Alterations in the p53 pathway in adenovirus-infected cells and tumours.
Vectors

- The way you insert the “normal” gene in the patient’s cell is by vectors.
- The most common vectors that are used in gene therapy are virus vectors.
Essential components of a gene therapy vector

- **A therapeutic gene**—that codes a therapeutic protein
- **A regulatory element**—to control the expression of the therapeutic gene
- **A gene delivery vehicle**—that can efficiently and accurately deliver the therapeutic gene into the target cells.
Gene delivery vectors

- **Viruses** *(retroviruses, adenoviruses, adeno associated viruses, etc)*
- **Non-viral vectors** *(naked DNA, plasmids, liposomes, packaged DNA particles etc)*
- **Cell therapies** *(stem cells: hematopoietic, mezanchymal, neuronal, embryonic)*
A survey of gene transfer clinical trials. a | Most gene-therapy clinical trials are designed to treat cancer. b | Retrovirus vectors and adenovirus vectors have, so far, been the most commonly used vectors in gene-transfer trials. Non-viral gene transfer has been assessed in roughly one-quarter of all trials.
Gene targeting

- **Physical targeting** (catheters, l.m. inj, micro spheres, electroporation, gene guns, etc)
- **Biological targeting** (transductional targeting, modifications viral capsid proteins or surface of the vectors)
- **Transcriptional targeting** (specific promoters)
Transductional targeting can be achieved by redirecting the vector capsid to new cellular receptors using molecular adaptors (usually bi-specific antibodies) that are conjugated to the capsid structure, or by genetically altering receptor-binding proteins in the virus capsid so that they recognize and bind to alternative receptors.
Combining transductional targeting with transcriptional targeting can further increase the efficacy and specificity of viral vector-mediated transduction.
Table I  Expression of CAR, $\alpha_\nu\beta_3$, $\alpha_\nu\beta_5$ Receptors and Tyrosinase in Cell Lines

FACS analysis was used to detect the percentage of CAR, $\alpha_\nu\beta_3$ and $\alpha_\nu\beta_5$ receptor positive cells. The data shown represents the mean $\pm$ SD (n=2). Tyrosinase transcripts were measured by RT-PCR.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>CAR</th>
<th>$\alpha_\nu\beta_3$</th>
<th>$\alpha_\nu\beta_5$</th>
<th>Tyrosinase transcripts</th>
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<td>MCF-7</td>
<td>0.75% $\pm$ 1%</td>
<td>4.8% $\pm$ 1%</td>
<td>77.4% $\pm$ 3%</td>
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<td>Hs695T</td>
<td>1.23% $\pm$ 2%</td>
<td>7.62% $\pm$ 2%</td>
<td>62.8% $\pm$ 2.5%</td>
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<tr>
<td>TF-2</td>
<td>4.8% $\pm$ 2%</td>
<td>37% $\pm$ 4%</td>
<td>38.8% $\pm$ 4%</td>
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<td>YUSAC2</td>
<td>4.9% $\pm$ 1%</td>
<td>78% $\pm$ 8%</td>
<td>18% $\pm$ 5%</td>
<td>positive</td>
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<tr>
<td>SK-Mel-28</td>
<td>85.8% $\pm$ 10%</td>
<td>47.2% $\pm$ 9%</td>
<td>28.6% $\pm$ 4%</td>
<td>positive</td>
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</tbody>
</table>
Transcriptional targeting is generally achieved by placing the transgene under the control of a cell-type-specific promoter.

Examples of promoters used for transcriptional targeting of gene therapy vectors:

- **Tissue-specific**
  - Tyrosinase
  - PSA
  - NSE
  - Albumin
- **Tumor-selective**
  - AFP
  - CEA
  - erbB2
  - L-plastin
- **Endothelium-specific**
  - KDR
  - E-selectin
- **Response directed**
  - Mdr-1
  - hsp70
- **Cell cycle directed**
  - E2F-1
  - Cyclin A, cdc25C
Strategies for Transgene Delivery

Ex Vivo

Cells removed from body

Transgene delivered

Cells cultured

Cells returned to the body

In Vivo

Transgene delivered directly into host
Routes of administration of gene therapy vectors

**Ex Vivo Approach**
- Harvested Cells or Tissue
- Culture
- 48hrs.
- Transfer of DNA
- Reinfusion of genetically altered cells

**In Vivo Approach**
- Construction of gene vectors
- Addition of therapeutic gene
- Recombinant vector
- Targeted delivery
- Systemic Infusion

1.  
2.  
3.  
Gene therapy of cancer

1. Conditionally replicating adenoviral vectors and suicide gene therapy
2. Vector targeting of chemotherapy (combination of CRADs and conventional chemotherapy)
3. Tumor specific immunotherapy with adenoviral vectors
4. Combination of suicide gene therapy and dendritic cells
5. Combination of tumor specific immunotherapy and chemotherapy/vector targeting of chemotherapy
Cytotoxic effect of replication competent adenoviral vectors carrying L-plastin promoter regulated E1A and cytosine deaminase genes in cancers of the breast, ovary and colon
Tumor volumes of the assigned treatment groups

Survival curves according to the Kaplan-Meier method of the assigned treatment groups
Vector targeting makes 5-fluorouracil chemotherapy less toxic and more effective in animal models of epithelial neoplasms.
Tumor volumes of the assigned treatment groups of Animal Model #1

Survival curves according to the Kaplan-Meier method of the assigned treatment groups of animal model #1:
- Group 1: AdLpCDIRESE1A/5FC/FA/CPT-11
- Group 2: AdLpCD/5FC/FA/CPT-11
- Group 3: AdLpCDIRESE1A/5FC
- Group 4: AdLpCDIRESE1A/5FC/FA
- Group 5: AdLpCD/5FC/FA
- Group 6: CPT-11/5FU/FA
- Group 7: AdLpCDIRESE1A/FA/CPT-11
- Group 8: Control
It is very difficult to eradicate the tumor cells in metastatic disease with any current therapeutic approach alone, including the experimental ones.
The immune system, though it is usually not sufficient when used alone, inherently has the potential of eradicating the metastatic tumor cells (esp. min res. dis).
Dendritic cells is the central to the regulation, maturation and maintenance of a cellular immune response to cancer.

Therefore, targeting DCs seems to be an efficient way of improving results of gene therapy.
Tumour antigens can be isolated and characterized from cDNA libraries or from peptides eluted from the tumour-cell surface. Dendritic cells (DCs) are loaded with tumour antigens. The antigen-loaded DCs are matured *ex vivo* by incubation with various agents, such as CRT, calreticulin; ds, double stranded; HSP, heat-shock proteins; IL-1, interleukin-1; LPS, lipopolysaccharide; PGE2, prostaglandin E2; TNF-α, tumour-necrosis factor to recapitulate the process occurring at the site of pathogen-induced inflammatory reaction.

1. Local infection of cells surrounding injection site
2. Release of the tumor antigen/CD40 ligand protein continuously over 10-14 days
3. Binding of the TAA/CD40 ligand protein to dendritic cells in the subcutaneous tissue
4. Activation of antigen presenting cells (The binding of the CD40 ligand to CD40 (a receptor) on dendritic cells can induce DC highly express MHC I, II, costimulatory and adhesion molecules, including B7-1, B7-2, CD40 and ICAM-1.)
5. Uptake of tumor antigen into antigen presenting cells
6. Migration of the activated dendritic cells to regional lymph nodes
7. Generation of CD8 T cells which are selectively cytolytic for cancer cells carrying the TAA (Mature DCs can induce activation and proliferation of naïve T-helper and T-cytotoxic lymphocytes (CTLs).
8. Generation of resistance for the engraftment of the TAA positive cancer cells in the mouse
DNA vaccine (viral vector) to induce costimulatory factors and cytokines

Dendritic cell vaccine – dendritic cells undergo viral transduction of more effectively present antigen
1.1.1. Testing the efficacy of a human adenoviral vector in mouse cells.
   a. Mouse tumor cell lines
   b. Mouse dendritic cells


3. Testing the in vitro cytotoxicity of AdCDIRESE1A adenoviral vector system, carrying a cytosine deaminase (CD) in mouse tumor cell lines.

4. Testing the specific immunity induced by the treatment.

5. Testing the in vivo efficacy of the combination of intratumoral injection of AdCDIRESE1A +5-FC system and Ad-sig-E7/ecdCD40L vector prime/E7/ecdCD40L protein boost.
The combination of intratumoral injections of the AdCDIRESE1A vector, intraperitoneal 5-FC, and concomitant s.c. injection of Ad-sig-E7/ecdCD40L vector prime/E7/ecdCD40L protein boost is more effective than either alone.

The combination of immunotherapy with vector targeted chemotherapy could have an impact on the control of disseminated cancer (esp in minimal residual disease).
Subcutaneous Injection of Adenoviral Vector Encoding a CD40Ligand/Tumor Antigen Secretory Protein Generates T Cell Dependent Cellular Immunity Against Tumor Cell Lines for Up to One Year

<table>
<thead>
<tr>
<th>SIG</th>
<th>HPV E7</th>
<th>NDAQAPK</th>
<th>Secretable CD40 ligand</th>
</tr>
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<td>SIG</td>
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<td>NDAQAPK</td>
<td>Secretable CD40 ligand</td>
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<td>HPV E7</td>
<td>NDAQAPK</td>
<td>Non-Secretable CD40 ligand</td>
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<tr>
<td>SIG</td>
<td>NDAQAPK</td>
<td>Secretable CD40 ligand</td>
<td></td>
</tr>
</tbody>
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Figure 2

1: Injection of vaccine

2: Binding to dendritic cell

3: E7 covers surface of DC

4: DC migrates to lymph node

5: DC expands T cells which can kill E7 positive cancer cells

6: T cells travel through blood to tumor nodule and kill E7 positive cancer cells

Dendritic cell (DC) is early warning cell of immune response

Tumor nodule

E7 marker

CD40L

Lymph nodes (location of immune response cells)
The binding of the CD40 ligand on B cells or CD4 T cells to CD40 (a receptor) on dendritic cells can induce DC highly express MHC I, II, costimulatory and adhesion molecules, including B7-1, B7-2, CD40 and ICAM-1. Then mature DCs preferentially migrate to the T cell areas of secondary lymphoid tissues where they can induce activation and proliferation of naïve T-helper and T-cytotoxic lymphocytes (CTLs), also CD40L induces IL-12 production, a cytokine that regulates Th cell differentiation into Th1 cells
Antigen specificity of resistance to growth of TC-1 Cells After S.C. vector injection

Immunity to growth of TC-1 cells persists 1 year after vector injection
1. Injection of Ad-CMV-sigE7/SCD40L vector induces resistance to E7 positive cell line
2. Resistance is dependent on T cells
3. Induction of resistance is dependent on signal secretory sequence for the E7/CD40 protein
4. Induction of resistance is dependent on CD40
5. Induction of cellular immunity to E7 positive cells persists for up to one year
6. Ad-CMV-sigE7/SCD40L vector could be used as a vaccine for HPV to prevent cervical cancer
7. Ad-CMV-sigTumorAntigen/SCD40L vector injection could be used to treat metastatic cancer
Combination of dendritic cells with vector targeted chemotherapy Improves treatment efficacy
In vivo efficacy of the combination of intratumoral injection of AdCDIRESE1A +5-FC system and dendritic cells.

The combination of intratumoral injections of the AdCDIRESE1A vector plus intraperitoneal 5-FC concomitantly with intratumoral injection DCs is more effective than either alone.

The combination of immunotherapy with vector targeted chemotherapy could have an impact on the control of disseminated cancer.
Experimental approaches to tumor therapy by immunization

- **Find tumor specific antigens** using antitumor CD8 T cells or antibodies.
- **Make tumors more immunogenic** by mixing them with adjuvants or forcing them to express cytokines or costimulatory molecules.
- **Reverse the natural processes that limit immune reactions.** (Blocking CTLA4, eliminating CD25+CD4+ suppressor cells)
- **Dendritic cell loading** with specific antigen or whole tumor cells and stimulation with specific adjuvants in cell culture prior to reintroduction to host.
• Cancer vaccines present different challenges and opportunities. Faced with life threatening disease, the possibility of using more powerful adjuvants and vaccines that may lead to autoreactivity are less of a limitation than in prophylactic vaccines. Major limitations involve the identification of robust antigens the elicit a strong, high affinity T cell response. Other barriers include the problem of chemotherapy induced immunosuppression and mutational escape of immune recognition by the tumors.
A. patient no. 2: left upper lobe tumor unable to undergo surgery because of poor pulmonary function and cardiac disease. Patient received three injections of Ad-p53 (3 \times 10^{11} \text{ vp}) via bronchoscope in combination with radiation therapy (60 Gy; A). Pathologic biopsy negative for viable tumor 3 months after completion of therapy (B). B, patient no. 3: right upper lobe tumor unable to be treated with surgery because of poor pulmonary function and ineligible for chemotherapy because of cardiac disease and obstructed bronchus (5/29/98). Patient was treated with three injections of Ad-p53 (3 \times 10^{11} \text{ vp}) and radiation therapy (60 Gy) by bronchoscopy (5/29/98) with a CR 3 months after completion of therapy (10/8/98) and no pathologic evidence of tumor 29 months after therapy (12/11/00).

57\% of the patients showed that the cancer progressed to worse stages. Why?
Major Problems to Overcome

- Identify more efficient ways to deliver the genes to the patients’ genetic material
- Develop vectors that can specifically focus on the targeted cells
- Ensure that vectors will successfully insert the desired genes into each of these target cells
- Deliver genes to a precise location in the patient’s DNA
- Ensure that transplanted genes are precisely controlled by the body’s normal physiologic signals